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     (FILE 'HOME' ENTERED AT 14:25:55 ON 09 AUG 2000)

     INDEX 'ADISALERTS, ADISINSIGHT, AGRICOLA, AIDSLINE, ANABSTR, AQUASCI,
     BIOBUSINESS, BIOCOMMERCE, BIOSIS, BIOTECHABS, BIOTECHDS, BIOTECHNO,
CABA,
     CANCERLIT, CAPLUS, CEABA, CEN, CIN, CONFSCI, CROPB, CROPU, DDFB, DDFU,
     DGENE, DRUGB, DRUGLAUNCH, DRUGMONOG2, ...' ENTERED AT 14:26:09 ON 09 AUG
     2000
               SEA BETA-SECRETASE
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                1   FILE ADISALERTS
                1   FILE ADISINSIGHT
                2   FILE BIOBUSINESS
                1   FILE BIOCOMMERCE
              166   FILE BIOSIS
                5   FILE BIOTECHABS
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               59   FILE BIOTECHNO
               24   FILE CANCERLIT
              144   FILE CAPLUS
                1   FILE CEABA
                3   FILE CEN
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               81   FILE DGENE
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                7   FILE EMBAL
              112   FILE EMBASE
               88   FILE ESBIOBASE
               14   FILE GENBANK
                4   FILE IFIPAT
                3   FILE JICST-EPLUS
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              100   FILE MEDLINE
                3   FILE PHAR
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              121   FILE SCISEARCH
               18   FILE TOXLINE
               11   FILE TOXLIT
               19   FILE USPATFULL
               10   FILE WPIDS
               10   FILE WPINDEX
L1               QUE BETA-SECRETASE
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     FILE 'MEDLINE, EMBASE, BIOSIS, SCISEARCH, CAPLUS, USPATFULL' ENTERED AT
     14:29:17 ON 09 AUG 2000
L2           662 S L1
L3           109 S L2 AND (CDNA OR CLON? OR POLYPEPTIDE(W)SEQUENC?)
L4            56 DUP REM L3 (53 DUPLICATES REMOVED)
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L4 ANSWER 46 OF 56 SCISEARCH COPYRIGHT 2000 ISI (R)
ACCESSION NUMBER: 97:692679 SCISEARCH
THE GENUINE ARTICLE: XV868
TITLE: Expression and characterization of human **beta-secretase** candidates metalloendopeptidase MP78 and cathepsin D in beta APP-overexpressing cells
AUTHOR: Thompson A; GrueningerLeitch F; Huber G; Malherbe P (Reprint)
CORPORATE SOURCE: F HOFFMANN LA ROCHE & CO LTD, PRECLIN CNS RES, DIV PHARMA,
BLDG 69-333, CH-4070 BASEL, SWITZERLAND (Reprint); F HOFFMANN LA ROCHE & CO LTD, PRECLIN CNS RES, DIV PHARMA, CH-4070 BASEL, SWITZERLAND; F HOFFMANN LA ROCHE & CO LTD, GENE TECHNOL, CH-4070 BASEL, SWITZERLAND
COUNTRY OF AUTHOR: SWITZERLAND
SOURCE: MOLECULAR BRAIN RESEARCH, (SEP 1997) Vol. 48, No. 2, pp. 206-214.
Publisher: ELSEVIER SCIENCE BV, PO BOX 211, 1000 AE AMSTERDAM, NETHERLANDS.
ISSN: 0169-328X.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: English
REFERENCE COUNT: 29

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Human **beta-secretase** candidates, MP78 (h-MP78, EC 3.4.24.15) and cathepsin D (Cat D, EC 3.4.23.5), were evaluated for their ability to enhance amyloid-beta-protein (A beta) secretion when overexpressed in beta APP-containing cells. HEK-293 cells stably co-expressing h-MP78 or Cat D and h-beta APP695 were metabolically labeled with [S-35]methionine and A beta secretion was quantified in the conditioned media by immunoprecipitation and ELISA without showing any significant increase in A beta production Because Cat D is known to have a higher affinity for APP-substrate containing the Swedish familial Alzheimer's disease double mutation (SFAD, K595N and M596L substitutions in beta APP695) than for the wild type substrate [Dreyer et al., fur. J. Biochem., 224 (1994) 265-271], the effect of Cat D overexpression was tested in a HEK293/beta APPSFAD stable cell line. ELISA analysis of the conditioned media from these cells did also not reveal any increase in A beta generation. In addition, recombinant h-MP78 purified from E, coli cleaved an APP-derived substrate spanning the **beta-secretase** site (ISEVKMD(1)AEFRHDS) at multiple sites, but the beta-site cleavage was only a minor one; cleavage occurred predominantly at K-M and E-F bonds. Human liver Cat D also cleaved the same substrate at multiple sites, yet the major cleavage at pH 4.0 occurred at the amyloidogenic D-1 site. These findings indicate that h-MP78 does not have the cleavage specificity required for a **beta-secretase** protease and although Cat D fulfilled the amyloidogenic cleavage specificity, the results of the co-expression experiments make both enzymes less likely candidates as relevant **beta-**

L4 ANSWER 45 OF 56 MEDLINE DUPLICATE 14
ACCESSION NUMBER: 97234570 MEDLINE
DOCUMENT NUMBER: 97234570
TITLE: A possible role for cathepsins D, E, and B in the processing of beta-amyloid precursor protein in Alzheimer's disease.
AUTHOR: Mackay E A; Ehrhard A; Moniatte M; Guenet C; Tardif C; Tarnus C; Sorokine O; Heintzelmann B; Nay C; Remy J M; Higaki J; Van Dorsselaer A; Wagner J; Danzin C; Mamont P
CORPORATE SOURCE: Marion Merrell Research Institute, Strasbourg, France.
SOURCE: EUROPEAN JOURNAL OF BIOCHEMISTRY, (1997 Mar 1) 244 (2) 414-25.
Journal code: EMZ. ISSN: 0014-2956.
PUB. COUNTRY: GERMANY: Germany, Federal Republic of
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; Cancer Journals
ENTRY MONTH: 199706

AB Formation of the 4-kDa peptides, which are essential constituents of the extracellular plaques in Alzheimer's disease, involves the sequential cleavage of the amyloid precursor protein (APP) by beta- and gamma-secretases. The carboxy-terminal 99-amino-acid peptide which is liberated from APP by **beta-secretase** was used as a potential native substrate of the gamma-secretase(s). With the addition of an initiator Met and a FLAG sequence at the C-terminus (betaAPP100-FLAG), it was expressed in Escherichia coli under the control of the T7 promotor.

The preferred site(s) of cleavage in the N-terminal 40-amino-acid beta-amyloid peptide and betaAPP100-FLAG by potential gamma-secretase(s) were rapidly identified using matrix-assisted laser-desorption/ionization time-of-flight mass spectroscopy in addition to peptide mapping followed by protein sequence analysis. Since gamma-secretases seem to be active at acidic pH, three cathepsins (D, E and B) were selected for testing. Studies using different detergents indicated that the cleavage preference of cathepsin D for the betaAPP100-FLAG is highly dependent on the surfactant used to solubilize this substrate. All three cathepsins were found to be capable of catabolizing both beta-amyloid peptides and the betaAPP100-FLAG. As cathepsin D was found to cleave the betaAPP100-FLAG in the vicinity of the C-terminus of the beta-amyloid peptides and cathepsin B has a high carboxypeptidase activity at low pH, the possibility cannot be excluded that cathepsins D and B are involved in the amyloidogenic processing of APP.

L4 ANSWER 35 OF 56 MEDLINE DUPLICATE 12
ACCESSION NUMBER: 1998453345 MEDLINE
DOCUMENT NUMBER: 98453345
TITLE: Processing of the Alzheimer's disease amyloid precursor protein in Pichia pastoris: immunodetection of alpha-, beta-, and gamma-secretase products.
AUTHOR: Le Brocque D; Henry A; Cappai R; Li Q X; Tanner J E; Galatis D; Gray C; Holmes S; Underwood J R; Beyreuther K; Masters C L; Evin G
CORPORATE SOURCE: Department of Pathology, The University of Melbourne, Parkville, Victoria, Australia.
SOURCE: BIOCHEMISTRY, (1998 Oct 20) 37 (42) 14958-65.
Journal code: A0G. ISSN: 0006-2960.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199901
AB betaA4 (Abeta) amyloid peptide, a major component of Alzheimer's disease (AD) plaques, is a proteolytic product of the amyloid precursor protein (APP). Endoproteases, termed beta- and gamma-secretase, release respectively the N- and C-termini of the peptide. APP default secretion involves cleavage within the betaA4 domain by alpha-secretase. To study the conservation of APP processing in lower eukaryotes, the yeast Pichia pastoris was transfected with human APP695 **cDNA**. In addition to the full-length integral transmembrane protein found in the cell lysate, soluble/secreted APP (sAPP) was detected in the culture medium. Most sAPP comprised the N-terminal moiety of betaA4 and corresponds to sAPPalpha, the product of alpha-secretase. The culture medium also contained minor secreted forms detected by a monoclonal antibody specific for sAPPbeta (the ectodomain released by **beta-secretase** cleavage).
Analysis of the cell lysates with specific antibodies also detected membrane-associated C-terminal fragments corresponding to the products of alpha and beta cleavages. Moreover, immunoprecipitation of the culture medium with three antibodies directed at distinct epitopes of the betaA4 domain yielded a 4 kDa product with the same electrophoretic mobility as betaA4 synthetic peptide. These results suggest that the alpha-, beta-, and gamma-secretase cleavages are conserved in yeast and that P. pastoris may offer an alternative to mammalian cells to identify the proteases involved in the generation of AD betaA4 amyloid.